Concept for a rapid point-of-care calprotectin diagnostic test for diagnosis and disease activity monitoring in patients with inflammatory bowel disease: expert clinical opinion

Rogler, Gerhard ; Aldeguer, Xavier ; Kruis, Wolfgang ; Lasson, Anders ; Mittmann, Ulrich ; Nally, Ken ; Peyrin-Biroulet, Laurent ; Schoepfer, Alain ; Vatn, Morten ; Vavricka, Stephan ; Logan, Robert

DOI: https://doi.org/10.1016/j.crohns.2013.02.014

Concept for a rapid point-of-care calprotectin diagnostic test for patients with organic and non-organic bowel disease: Expert clinical opinion

G. Rogler[1], X. Aldeguer[2], W. Kruis[3], A. Lasson[4], U. Mittmann[5], K. Nally[6], L. Peyrin-Biroulet[7], A. Schoepfer[8], T. Sipponen[9], M. Vatn[10], S. Vavricka[11], R. Logan[12]

[1]Department of Medicine, University Hospital of Zürich, Zürich, Switzerland; [2]Hospital Doctor Josep Trueta, Girona, Catalonia, Spain; [3]Evangelisches Krankenhaus Kalk, Innere Medizin, University of Cologne, Germany; [4]Department of Internal Medicine, Södra Alvsborgs Hospital, Borås, Sweden; [5]Mittmann Pharma Consulting, Basel, Switzerland; [6]Alimentary Pharmabiotic Centre, University College Cork, National University of Ireland, Cork, Ireland; [7]Department of Gastroenterology, Inserm U954, University Hospital of Nancy, Henri Poincaré University, Vandœuvre-lès-Nancy, France; [8]Department of Gastroenterology and Hepatology, CHUV/University Hospital Lausanne, Lausanne, Switzerland; [9]Department of Medicine, Division of Gastroenterology, Helsinki University Central Hospital, Helsinki, Finland; [10]University of Oslo, EpiGen, Campus Ahus and Section for Gastroenterology, Oslo University Hospital, Rikshospitalet, Oslo, Norway; [11]Division of Gastroenterology and Hepatology, Triemli Hospital Zürich, Zürich, Switzerland; [12]Kings College Hospital NHS Foundation Trust, Kings College Hospital, London, United Kingdom

Corresponding author: Gerhard Rogler, Division of Gastroenterology and Hepatology, Department of Medicine, University Hospital of Zürich, Zürich Center for Integrative Human Physiology (ZIHP), University of Zürich, Rämistrasse 100, 8091 Zürich, Switzerland. E-mail: gerhard.rogler@usz.ch. Telephone number: +41 (0)44 255 9477. Fax number: +41 (0)44 255 9497.

Short running title: Rapid calprotectin diagnostic test for patients with IBD

Key words: calprotectin, Crohn's disease, diagnostic test, IBD, IBS, inflammatory bowel disease, irritable bowel syndrome, ulcerative colitis

Word count: 3,428 (not including references or table titles)

Summary

Background: Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD) is characterized in patients by repeated periods of disease activity and remission. In recent years, fecal biomarkers have been evaluated for their ability to aid diagnosis of IBD, differentiate IBD from irritable bowel syndrome (IBS), assess IBD activity and estimate prognosis.

Aim: An expert panel met to discuss the development of a rapid point-of-care semi-quantitative test for discrimination of IBD from IBS and subsequent disease activity monitoring in patients with IBD.

Methods: Eleven gastroenterologists with expertise in IBS/IBD met in April 2012 to discuss and agree the most suitable biomarker for a rapid point-of-care semi-quantitative test and to recommend the number and cut-off level values in various clinical settings.

Results: Fecal calprotectin was agreed as the most suitable biomarker for the point-of-care test. Four clinical situations benefitting from a rapid diagnostic test were identified and calprotectin cut-off levels were proposed for each: early diagnosis of IBD and discrimination from IBS in primary and/or secondary care settings (cut-off level of 100 µg/g), detection and prediction of IBD flares whilst in remission (250 µg/g) and monitoring of active IBD to optimize treatment (400 µg/g), with 1000 µg/g recommended for diagnosing severe active disease.

Conclusion: An expert panel of gastroenterologists recommended four clinical settings and corresponding cut-off fecal calprotectin values for the development of a rapid point-of-care semi-quantitative test for discrimination of IBD from IBS, the detection and prediction of IBD flares during remission and monitoring of active and severe active IBD disease.

Introduction

Inflammatory bowel diseases (IBD), such as ulcerative colitis (UC) and Crohn's disease (CD) are chronic, organic inflammatory diseases, caused by acute mucosal inflammation of the intestine. They are typically characterized by variable disease activity, often with repeated periods of intermittent disease activity and remission. Irritable bowel syndrome (IBS) is a non-inflammatory functional disorder and by definition, presumes the absence of organic disease.[1] As the presenting manifestations of IBD and IBS are similar and can include diarrhea, abdominal pain and bloating, obtaining a clinical diagnosis can be difficult and further invasive diagnostic procedures may be required in order to obtain a confirmed diagnosis.[2, 3]

Treatment strategies based on presenting clinical manifestations have failed to modify the course of IBD.[4, 5] Indeed clinical disease activity indices are non-specific and do not correlate with endoscopic activity.[2, 5-7]

Mucosal healing has emerged as a new therapeutic goal in clinical practice and is regarded as a major endpoint definition for remission of IBD in clinical trials, due to its association with improved outcomes in IBD, including reduced relapse and hospitalization rates and reduced need for surgery.[8] C-reactive protein (CRP) is a non-specific marker that reflects systemic inflammation and is useful for the detection of complications (such as abscesses) or extraintestinal manifestations.[9-14] However, it is not an accurate marker of mucosal healing.[15]

There is currently an unmet medical need for a suitable non-invasive biomarker of mucosal inflammation, arising primarily because of the inherent problems associated with endoscopy, which is an invasive and expensive procedure.

Although cross-sectional imaging methods such as MRI[16, 17], CT and ultrasound are used for assessing disease activity in patients with UC and CD, there is still room for the use of fecal markers to evaluate mucosal lesions in clinical practice. In addition, a non-invasive test might be used to anticipate flares in IBD activity which would allow for pre-emptive escalation of treatment. The ideal marker should be specific and sensitive, inexpensive, yield rapid results, which could be available during the patient visit, and have a high negative predictive value.

This article reports the outcomes of a recent meeting of a panel of gastroenterologists with expertise in IBD and fecal biomarkers to discuss the development of a rapid point-of-care semi-quantitative test to enable discrimination of IBD from IBS and subsequent disease activity monitoring in patients with IBD.

Methods

In April 2012, 11 gastroenterologists with expertise in IBD and special interest in fecal biomarkers met in Zürich, Switzerland to discuss the potential for development of a rapid point-of-care, semi-quantitative test. The meeting focused on the following main questions:

1. What is the rationale for choosing fecal calprotectin as the most suitable IBD biomarker for the test, compared with other fecal markers such as lactoferrin or M2-pyruvate kinase?
2. What would be the agreed number and cut-off calprotectin level values for various clinical settings?
3. Would it be useful to have different cut-off levels for UC and CD?
4. Could patients with IBD tailor their treatment to allow earlier escalation of their treatments to prevent a relapse of their disease or, alternatively, reduce their treatment if their disease was known to be in remission as indicated by the results of their fecal marker rapid point-of-care test?

Finally, the expert panel discussed next steps in terms of a clinical study concept to validate agreed consensus calprotectin cut-off levels.

Results

Candidate markers for a rapid point-of-care diagnostic test

Inflammation of the intestine during the acute-phase IBD is associated with migration of leukocytes to the gut, resulting in the production of a large number of inflammatory proteins which can be measured in the feces. As a result, several leukocyte products and serum proteins have been evaluated for use as fecal markers, which can be used to indicate the presence and severity of intestinal inflammation.[10, 18] Possible fecal markers for IBD include lactoferrin, M2-pyruvate kinase, S100A12, calprotectin and polymorphonuclear neutrophil elastase (PMN-elastase). The characteristics of these IBD markers are summarized in Table 1.

Lactoferrin is an iron-binding glycoprotein found in neutrophil granulocytes; its levels in feces have been shown to increase quickly following inflammation of the intestine.[18] Studies have reported that lactoferrin is a sensitive marker of IBD activity[19-21]; however, it is not as widely studied as calprotectin. Walker *et al.* reported promising results from a study of 148 children and young adults with IBD (141 with IBD and 7 with IBS, plus 22 healthy controls) in which elevated lactoferrin levels were used to identify patients at greater risk of disease relapse.[22]

M2-pyruvate kinase (M2-PK) is an enzyme involved in glycolysis that is present in rapidly dividing cells and has been previously used as a marker of gastrointestinal cancers.[23] However, its role in gastrointestinal inflammation is unknown. A study by Chung-Faye *et al.* reported elevated concentrations of M2-PK in patients with IBD compared with those patients with IBS. However, it

has yet to be shown if M2-PK can be used as a potential marker for predicting relapses in asymptomatic patients with IBD.[24]

S100A12 (calgranulin C), a member of the human calcium-binding S100 protein family, is a cytoplasmic protein found in neutrophils that acts as a marker of inflammation.[23] Previous studies carried out in both children and adults reported positive results regarding the ability of S100A12 to distinguish between IBD and IBS.[25] However, it has not yet been determined whether S100A12 could be useful as a marker of disease relapse in both the pediatric and adult setting.[23]

Calprotectin is a heterocomplex of S100A8 (migration inhibitory factor-related protein (MRP) 8, calgranulin A) and S100A9 (MRP14, calgranulin B), two other members of the calcium-binding S100 protein family.[23] Fecal calprotectin represents up to 60% of cytosolic protein in neutrophils and levels correlate with the influx of neutrophils into the intestine.[18] Calprotectin is stable in feces for up to 7 days at room temperature and this property offers advantages in both clinical practice and in terms of a suitable marker for a diagnostic test.[26]

PMN-elastase is a serine proteinase that is secreted by neutrophils and macrophages during inflammation and the concentrations of PMN-elastase in feces correlate with intestinal inflammation. A study by Schröder *et al.* identified the use of PMN-elastase as a possible tool for the differential diagnosis of IBS; however, its diagnostic accuracy is lower than that of lactoferrin and calprotectin.[27]

The predictive value of biomarkers in the diagnosis and treatment of patients with IBD has been more widely explored for calprotectin than the other biomarkers mentioned here.[23] Of particular interest is the potential for

calprotectin levels to be used to diagnose IBD,[28] differentiate IBD from IBS,[29, 30] assess IBD activity, and predict relapse of active disease.[31-36] Several clinical studies have demonstrated that not only does fecal calprotectin significantly correlate with endoscopic disease activity in active IBD,[15, 28, 37-40] but also that a normalization of fecal calprotectin equates to mucosal healing in IBD, which is an important treatment target goal.[41-45] Studies have shown that fecal calprotectin levels more closely correlate with endoscopic disease findings than CRP, blood leucocytes, CDEIS and CDAI.[15, 38] A report commissioned by the UK Department of Health in 2009 found fecal calprotectin to be more cost effective and accurate in discriminating between IBS and IBD in primary care in comparison to other inflammatory markers of IBD, such as CRP.[46] In addition, the UK National Institute for Health and Clinical Excellence is currently developing guidance on the application of fecal calprotectin as a diagnostic test for the differentiation of IBD from IBS.[47] Use of fecal calprotectin for diagnosing IBD could lead to a reduction in the number of specialist referrals for further invasive endoscopic investigations. A meta-analysis of 13 prospective diagnostic accuracy studies (6 studies involving 670 adults with fecal calprotectin cut-off levels ranging from 24 µg/g to 150 µg/g; and 7 studies involving 371 children with fecal calprotectin cut-off levels ranging from 32 µg/g to 100 µg/g) predicted that screening with fecal calprotectin to exclude IBD could hypothetically reduce the number of endoscopy referrals by 67% in adults and by 35% in children.[48]

On the basis of the current available evidence, the expert panel agreed that fecal calprotectin would be the best potential biomarker for a rapid point-of-care diagnostic test.

Currently, most assays for fecal calprotectin are based on the enzyme-linked immunosorbent assay (ELISA) method, which can be associated with a delay of up to 3 weeks before obtaining the results.[31] However, the ELISA method provides a quantitative result, which might be of additional clinical value. Previous studies have shown that there is some variability in calprotectin values both between patients and between samples; therefore, the clinical value of quantitative data remains to be determined.[29, 35, 40, 49] Development of a rapid, semi-quantitative, point-of-care test would dramatically reduce the turnaround time for test results to be available to physicians, thus allowing for a faster decision to be made regarding the current IBD status of a patient and the subsequent suitable treatment options.

An ideal rapid point-of-care diagnostic test needs to be easy to perform, accurate, specific and sensitive, and yield rapid results at the point-of-care, to allow the test results to be available during the patient visit.

Use of a semi-quantitative rapid point-of-care calprotectin test in clinical settings

The expert panel reached a consensus that a semi-quantitative fecal calprotectin point-of-care test would be useful in several IBD clinical settings: at initial diagnosis (discrimination between organic [IBD] and non-organic disease [IBS]); monitoring of patients with chronic IBD currently in remission; and monitoring of therapy efficacy in patients with active and severe active disease. The expert panel also agreed on the number and range of cut-off levels for each setting depending on the intended use (Table 2).

Kommentar [JB1]: Dear authors, previous drafts of the manuscriot stated that a summary of the results reported in three recent abstracts comparing the Bühlmann and immunodiagnostic ELISA test would be incorporated.

I have been unable to obtain the abstracts from the AGA and UEGW meetings (as per Dr Logan's suggestion).

Given the current manuscript word count of 3,428, please could you advise if this information is essential for inclusion in the manuscript?

Diagnosis of IBD and discrimination from IBS in primary and/ or secondary care settings

An important goal of a semi-quantitative point-of-care test would be the non-invasive discrimination of IBD from IBS. This point-of-care test would be ideally placed for use during the early diagnosis stage in primary care but it could also be used in the secondary care setting, as well as for differentiation of symptomatic patients with coexisting IBD and IBS. Discriminating possible IBD from IBS at an early stage would avoid unnecessary specialist referral of patients and subsequent endoscopic investigations. A single cut-off level of 100 μg/g was agreed by the expert panel as appropriate for this purpose based on results from previous studies which reported increased diagnostic precision for discrimination of colorectal inflammation in patients with CD and UC at 100 μg/g.[50, 51] This agreed cut-off level of 100 μg/g is higher than the levels suggested in recent studies, in which cut-off values of 30 to 50 μg/g were recommended.[28, 29, 37, 40, 46] It was agreed at the expert meeting that a higher cut-off level would be desirable to maximize the negative predictive value of the test to reduce incorrect diagnoses of IBD. As indicated in Table 2, a negative test result at the lowest cut-off level suggests a diagnosis of a non-inflammatory condition, such as IBS. A positive test result at the cut-off level of 100 μg/g may indicate IBS, with a recommendation to repeat the test in 6 weeks to confirm the initial result. However, a normal fecal calprotectin level alone would not be sufficient to confirm a diagnosis of IBS, without diagnostic criteria, such as Rome III being met. Positive results at both the 100 μg/g and 250 μg/g cut-off levels may indicate IBD rather than IBS.[1]

Detection and prediction of IBD flares whilst in remission

Another important clinical setting and goal for development of a semi-quantitative test was management of patients with established chronic IBD currently in remission. For patients exhibiting mild or moderate IBD symptoms, a corresponding increase in fecal calprotectin levels (as indicated by a positive point-of-care test result, Table 1) would be used to confirm/predict an active flare of IBD and suggest to physicians that a change of treatment or dose adjustment is required, without the need for further endoscopic investigations. For patients who are well, with minimal or no IBD symptoms, a point-of-care test with one or two cut-off values would be used to indicate and confirm that the disease is in a period of remission. It was recommended by the expert panel that both positive and negative test results should ideally be repeated to confirm the test result.

A fecal calprotectin value of 250 μg/g was deemed appropriate as a cut-off value for monitoring IBD remission based on results from previous studies. D'Haens *et al.* reported the results of a study which included 126 patients with IBD (87 patients with CD and 39 patients with UC) and proposed a fecal calprotectin cut-off value of 250 μg/g for indicating IBD remission.[37] An earlier study by Sipponen *et al.*, which included 77 patients with CD (106 endoscopies) proposed a cut-off value of 200 μg/g for identification of endoscopically inactive disease.[38] In another study of 115 patients with CD, fecal calprotectin levels less than 300 μg/g were associated with a reduced risk of disease relapse.[34]

Monitoring of active IBD to optimize treatment

Finally, the use of a point-of-care fecal calprotectin test would help assess therapy efficacy and optimize treatment in patients with active and severe active IBD by monitoring a reduction in fecal calprotectin levels. Fecal calprotectin levels are a sensitive measure of therapeutic efficacy and test results would aid physicians with treatment modification decisions. A cut-off calprotectin value of 400 µg/g was agreed by the expert panel with a further cut-off level of 1000 µg/g proposed for monitoring therapy efficacy in patients with severe active IBD. These two values were selected by the panel based on data from previously published studies which report a range of mean and median fecal calprotectin values of 175 µg/g,[37] 465 µg/g,[37] 718 µg/g,[15] 810 µg/g[52] and 1260 µg/g[53] in patients with active and severe active UC and CD.

Discussion

Experts agreed that a semi-quantitative fecal calprotectin test will be useful in clinical practice settings. The suggested cut-off values agreed by the expert panel are intended for a point-of-care test for use initially in patients with UC. It remains to be established if these proposed cut-off values can be used for patients with CD as the evidence base for interpreting fecal calprotectin results in patients with CD is less robust, which is partly due to the greater variety of disease manifestations in patients with CD.

There are some limitations to using fecal calprotectin as a proposed biomarker for monitoring disease activity in patients with IBD. Fecal calprotectin is a non-specific disease biomarker and whilst it is useful for discriminating IBD (organic disease) from IBS (non-organic disease), it is not useful for discriminating UC from CD or to discriminate active IBD from infectious gastrointestinal disease. Other presenting conditions such as non-steriodal anti-inflammatory drugs, tumors, and diverticulitis which cause an increase in intestinal neutrophils will result in increased concentrations of fecal calprotectin. Fecal calprotectin is more sensitive for detection of mild mucosal inflammation than CRP. In severely active cases, CRP better reflects systemic inflammation.[12, 14, 29]

Currently available tests can also yield results that vary substantially between and within patient samples, particularly in patients with CD.[34, 40, 49, 54] Results can also vary depending on the mixing, consistency and quantity of the stool sample, necessitating a standardized sample collection and preparation method to ensure test results are as accurate as possible.[55] Due to this variability in test results,

individual values can sometimes be difficult to interpret and it may be more appropriate to look at gross changes in fecal calprotectin levels i.e., a semi-quantitative rather than a quantitative test.

Semi-quantitative tests are fast, cheap, and may have the potential to be performed at home by the patients themselves or in an outpatient setting during patient visits. Home-tests are currently being used by patients with UC in Norway who are concerned that they may be having a flare.[49] An easy-to-use semi-quantitative test would offer practical advantages in this setting, enabling home-based testing to become more widely used for the monitoring of IBD. This would be of particular benefit to patients who are located some distance from medical centers or hospitals. It would also provide patients with a higher degree of ownership and sense of control over their condition. However, the expert panel agreed that both patient education and a good physician-patient relationship would be key support requirements needed to ensure correct usage and good compliance of a home-based semi-quantitative test, to ensure a benefit for patients. As the involvement of General Practitioners (GPs) in the diagnosis and treatment of IBD varies greatly between European countries, GPs do not always have the opportunity to provide their patients with this necessary support.

The expert panel agreed that a benchmarking study should be undertaken comparing the rapid point-of-care test against the standard ELISA test. This proof-of-concept study should evaluate the impact of the point-of-care test on the doctor's decision-making process and any potential improvement in patient's health-related quality of life, measured, for example, by reduced hospitalization of patients and a reduction in the number of invasive endoscopies. The

prognostic value of the point-of-care test should be included as a study endpoint measure.

In addition, larger prospective studies were suggested to be carried out to validate the cut-off values in clinical practice in patients with UC, using endoscopy as a reference for monitoring intestinal inflammation. Once cut-off values have been established for patients with UC, cut-off values for patients with CD could then be developed and validated, although cut-off values in CD will be harder to establish due to lack of consistent evidence. In addition, small intestinal disease is only reflected to a variable extent by fecal calprotectin values in contrast to colon involvement.[28, 56] At the time of preparing this paper, the UK National Institute for Health Research Health Technology Assessment Programme is welcoming applications from researchers to develop and validate a fecal biomarker tool, which can be used in primary and secondary care, for early detection of relapse in patients with CD.[57]

Conclusions

As a sensitive biomarker of mucosal inflammation, fecal calprotectin could be used in a rapid point-of-care test to monitor disease activity in patients with IBD and help reduce unnecessary specialist referrals and subsequent endoscopic investigations, which can be both uncomfortable and painful for the patient and time-consuming and costly for health services. As one of the main limitations of current calprotectin tests is the slow turnaround of results, a rapid semi-quantitative test would be an important development. This article reports the consensus opinion of an expert panelof gastroenterologist with expertise in IBD and highlights four clinical situations whereby the use of a rapid semi-quantitative test could be helpful, with recommended fecal calprotectin cut-off values proposed for each situation: early diagnosis and discrimination of IBD from other diseases such as IBS in primary and/or secondary care settings (cut-off level of 100 µg/g); detection and prediction of IBD flares in patients currently in remission (cut-off level of 250 µg/g); monitoring of active IBD (cut-off level of 450 µg/g); and severe active IBD (cut-off level of 1000 µg/g) to optimize treatment efficacy. Large prospective studies will be required to validate the recommended cut-off values in clinical practice.

References

1. Drossman DA. The functional gastrointestinal disorders and the Rome III process. *Gastroenterology* 2006; **130**(5): 1377–90.

2. Gomes P, du Boulay C, Smith CL, Holdstock G. Relationship between disease activity indices and colonoscopic findings in patients with colonic inflammatory bowel disease. *Gut* 1986; **27**(1): 92–5.

3. Quigley EM, Bernstein CN. Editorial: "irritable bowel symptoms" in inflammatory bowel disease: diagnostic uncertainty meets pathological reality. *Am J Gastroenterol* 2012; **107**(10): 1483–5.

4. Bouguen G, Peyrin-Biroulet L. Surgery for adult Crohn's disease: what is the actual risk? *Gut* 2011; **60**(9): 1178–81.

5. Filippi J, Allen PB, Hebuterne X, Peyrin-Biroulet L. Does anti-TNF therapy reduce the requirement for surgery in ulcerative colitis? A systematic review. *Curr Drug Targets* 2011; **12**(10): 1440–7.

6. Baars JE, Nuij VJ, Oldenburg B, Kuipers EJ, van der Woude CJ. Majority of patients with inflammatory bowel disease in clinical remission have mucosal inflammation. *Inflamm Bowel Dis* 2012; **18**(9): 1634–40.

7. Jorgensen LG, Fredholm L, Hyltoft Petersen P, Hey H, Munkholm P, Brandslund I. How accurate are clinical activity indices for scoring of disease activity in inflammatory bowel disease (IBD)? *Clin Chem Lab Med* 2005; **43**(4): 403–11.

8. Peyrin-Biroulet L, Ferrante M, Magro F*, et al.* Results from the 2nd Scientific Workshop of the ECCO. I: Impact of mucosal healing on the course of inflammatory bowel disease. *J Crohns Colitis* 2011; **5**(5): 477–83.

9. Mendoza JL, Abreu MT. Biological markers in inflammatory bowel disease: practical consideration for clinicians. *Gastroenterol Clin Biol* 2009; **33 Suppl 3**: S158–73.

10. Solem CA, Loftus EV, Jr., Tremaine WJ, Harmsen WS, Zinsmeister AR, Sandborn WJ. Correlation of C-reactive protein with clinical, endoscopic, histologic, and radiographic activity in inflammatory bowel disease. *Inflamm Bowel Dis* 2005; **11**(8): 707–12.

11. af Bjorkesten CG, Nieminen U, Turunen U, Arkkila P, Sipponen T, Farkkila M. Surrogate markers and clinical indices, alone or combined, as indicators for endoscopic remission in anti-TNF-treated luminal Crohn's disease. *Scand J Gastroenterol* 2012; **47**(5): 528–37.

12. Ricanek P, Brackmann S, Perminow G*, et al.* Evaluation of disease activity in IBD at the time of diagnosis by the use of clinical, biochemical, and fecal markers. *Scand J Gastroenterol* 2011; **46**(9): 1081–91.

13. Vermeire S, Van Assche G, Rutgeerts P. C-reactive protein as a marker for inflammatory bowel disease. *Inflamm Bowel Dis* 2004; **10**(5): 661–5.

14. Pettit SH, Holbrook IB, Irving MH. Comparison of clinical scores and acute phase proteins in the assessment of acute Crohn's disease. *Br J Surg* 1985; **72**(12): 1013–6.

15. Schoepfer AM, Beglinger C, Straumann A*, et al.* Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. *Am J Gastroenterol* 2010; **105**(1): 162–9.

16. Panes J, Ricart E, Rimola J. New MRI modalities for assessment of inflammatory bowel disease. *Gut* 2010; **59**(10): 1308–9.

17. Rimola J, Rodriguez S, Garcia-Bosch O*, et al.* Magnetic resonance for assessment of disease activity and severity in ileocolonic Crohn's disease. *Gut* 2009; **58**(8): 1113–20.

18. Desai D, Faubion WA, Sandborn WJ. Review article: biological activity markers in inflammatory bowel disease. *Aliment Pharmacol Ther* 2007; **25**(3): 247–55.

19. Dai J, Liu WZ, Zhao YP, Hu YB, Ge ZZ. Relationship between fecal lactoferrin and inflammatory bowel disease. *Scand J Gastroenterol* 2007; **42**(12): 1440–4.

20. Kane SV, Sandborn WJ, Rufo PA*, et al.* Fecal lactoferrin is a sensitive and specific marker in identifying intestinal inflammation. *Am J Gastroenterol* 2003; **98**(6): 1309–14.

21. Langhorst J, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. *Am J Gastroenterol* 2008; **103**(1): 162–9.

22. Walker TR, Land ML, Kartashov A*, et al.* Fecal lactoferrin is a sensitive and specific marker of disease activity in children and young adults with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2007; **44**(4): 414–22.

23. Judd TA, Day AS, Lemberg DA, Turner D, Leach ST. Update of fecal markers of inflammation in inflammatory bowel disease. *J Gastroenterol Hepatol* 2011; **26**(10): 1493–9.

24. Chung-Faye G, Hayee B, Maestranzi S, Donaldson N, Forgacs I, Sherwood R. Fecal M2-pyruvate kinase (M2-PK): a novel marker of intestinal inflammation. *Inflamm Bowel Dis* 2007; **13**(11): 1374–8.

25. Kaiser T, Langhorst J, Wittkowski H*, et al.* Faecal S100A12 as a non-invasive marker distinguishing inflammatory bowel disease from irritable bowel syndrome. *Gut* 2007; **56**(12): 1706–13.

26. Joishy M, Davies I, Ahmed M*, et al.* Fecal calprotectin and lactoferrin as noninvasive markers of pediatric inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2009; **48**(1): 48–54.

27. Schroder O, Naumann M, Shastri Y, Povse N, Stein J. Prospective evaluation of faecal neutrophil-derived proteins in identifying intestinal inflammation: combination of parameters does not improve diagnostic accuracy of calprotectin. *Aliment Pharmacol Ther* 2007; **26**(7): 1035–42.

28. Jensen MD, Kjeldsen J, Nathan T. Fecal calprotectin is equally sensitive in Crohn's disease affecting the small bowel and colon. *Scand J Gastroenterol* 2011; **46**(6): 694–700.

29. Schoepfer AM, Trummler M, Seeholzer P, Seibold-Schmid B, Seibold F. Discriminating IBD from IBS: comparison of the test performance of fecal markers, blood leukocytes, CRP, and IBD antibodies. *Inflamm Bowel Dis* 2008; **14**(1): 32–9.

30. Tibble JA, Sigthorsson G, Foster R, Forgacs I, Bjarnason I. Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. *Gastroenterology* 2002; **123**(2): 450–60.

31. Abraham BP, Kane S. Fecal markers: calprotectin and lactoferrin. *Gastroenterol Clin North Am* 2012; **41**(2): 483–95.

32. Garcia-Sanchez V, Iglesias-Flores E, Gonzalez R*, et al.* Does fecal calprotectin predict relapse in patients with Crohn's disease and ulcerative colitis? *J Crohns Colitis* 2010; **4**(2): 144–52.

33. Gisbert JP, Bermejo F, Perez-Calle JL*, et al.* Fecal calprotectin and lactoferrin for the prediction of inflammatory bowel disease relapse. *Inflamm Bowel Dis* 2009; **15**(8): 1190–8.

34. Louis E, Mary JY, Vernier-Massouille G*, et al.* Maintenance of remission among patients with Crohn's disease on antimetabolite therapy after infliximab therapy is stopped. *Gastroenterology* 2012; **142**(1): 63–70.

35. Tibble JA, Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. *Gastroenterology* 2000; **119**(1): 15–22.

36. Mao R, Xiao YL, Gao X*, et al.* Fecal calprotectin in predicting relapse of inflammatory bowel diseases: A meta-analysis of prospective studies. *Inflamm Bowel Dis* 2012; **18**(10): 1894–9.

37. D'Haens G, Ferrante M, Vermeire S*, et al.* Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. *Inflamm Bowel Dis* 2012 Feb 16. doi: 10.1002/ibd.22917.

38. Sipponen T, Savilahti E, Kolho KL, Nuutinen H, Turunen U, Farkkila M. Crohn's disease activity assessed by fecal calprotectin and lactoferrin: correlation

with Crohn's disease activity index and endoscopic findings. *Inflamm Bowel Dis* 2008; **14**(1): 40–6.

39. Sugi K, Saitoh O, Hirata I, Katsu K. Fecal lactoferrin as a marker for disease activity in inflammatory bowel disease: comparison with other neutrophil-derived proteins. *Am J Gastroenterol* 1996; **91**(5): 927–34.

40. Tibble J, Teahon K, Thjodleifsson B*, et al.* A simple method for assessing intestinal inflammation in Crohn's disease. *Gut* 2000; **47**(4): 506–13.

41. Jahnsen J, Roseth AG, Aadland E. [Measurement of calprotectin in faeces]. *Tidsskr Nor Laegeforen* 2009; **129**(8): 743–5.

42. Roseth AG, Aadland E, Grzyb K. Normalization of faecal calprotectin: a predictor of mucosal healing in patients with inflammatory bowel disease. *Scand J Gastroenterol* 2004; **39**(10): 1017–20.

43. Sipponen T, Bjorkesten CG, Farkkila M, Nuutinen H, Savilahti E, Kolho KL. Faecal calprotectin and lactoferrin are reliable surrogate markers of endoscopic response during Crohn's disease treatment. *Scand J Gastroenterol* 2010; **45**(3): 325–31.

44. Sipponen T, Savilahti E, Karkkainen P*, et al.* Fecal calprotectin, lactoferrin, and endoscopic disease activity in monitoring anti-TNF-alpha therapy for Crohn's disease. *Inflamm Bowel Dis* 2008; **14**(10): 1392–8.

45. Wagner M, Peterson CG, Ridefelt P, Sangfelt P, Carlson M. Fecal markers of inflammation used as surrogate markers for treatment outcome in relapsing inflammatory bowel disease. *World J Gastroenterol* 2008; **14**(36): 5584–9.

46. Pearson S, Whitehead S, Hutton J. Evidence review Value of calprotectin in screening out irritable bowel syndrome: NHS Purchasing and Supply Agency January 2010.

47. National Institute for Health and Clinical Excellence. Faecal calprotectin diagnostic tests to differentiate inflammatory bowel disease from irritable bowel syndrome. Available at: http://guidance.nice.org.uk/DT/12. Accessed on: 30 October 2012.

48. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. *BMJ* 2010; **341**: c3369.

49. Moum B, Jahnsen J, Bernklev T. Fecal calprotectin variability in Crohn's disease. *Inflamm Bowel Dis* 2010; **16**(7): 1091–2.

50. Limburg PJ, Ahlquist DA, Sandborn WJ*, et al.* Fecal calprotectin levels predict colorectal inflammation among patients with chronic diarrhea referred for colonoscopy. *Am J Gastroenterol* 2000; **95**(10): 2831–37.

51. von Roon AC, Karamountzos L, Purkayastha S*, et al.* Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am J Gastroenterol* 2007; **102**(4): 803–13.

52. Molander P, Af Bjorkesten CG, Mustonen H*, et al.* Fecal calprotectin concentration predicts outcome in inflammatory bowel disease after induction therapy with TNFalpha blocking agents. *Inflamm Bowel Dis* 2012; **18**(11): 2011–7.

53. De Vos M, Dewit O, D'Haens G*, et al.* Fast and sharp decrease in calprotectin predicts remission by infliximab in anti-TNF naive patients with ulcerative colitis. *J Crohns Colitis* 2012; **6**(5): 557–62.

54. Roseth AG, Schmidt PN, Fagerhol MK. Correlation between faecal excretion of indium-111-labelled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. *Scand J Gastroenterol* 1999; **34**(1): 50–4.

55. Vestergaard TA, Nielsen SL, Dahlerup JF, Hornung N. Fecal calprotectin: assessment of a rapid test. *Scand J Clin Lab Invest* 2008; **68**(4): 343–7.

56. Costa F, Mumolo MG, Ceccarelli L*, et al.* Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. *Gut* 2005; **54**(3): 364–8.

57. National Institute for Health Research Health Technology Assessment Programme. The early identification of relapse in Crohn's disease. Available at: http://www.hta.ac.uk/funding/standardcalls/12_143cb.pdf. Accessed on: 30 October 2012.

Tables

Table 1. Characteristics of existing IBD markers

Criteria for comparison	Lactoferrin	M2-pyruvate kinase	S100A12	Calprotectin	PMN-elastase
Structure	Iron-binding glycoprotein	Key enzyme in the glycolytic pathway	Cytoplasmic protein	Calcium-binding protein, heterocomplex of S100A8 and S100A9 proteins	Glycoprotein belonging to the group of serine proteinases
Derived from	Secondary granules of polymorpho-nuclear neutrophils	Expressed by proliferating tissues	Neutrophils	Mainly from neutrophils, occasionally from monocytes and reactive macrophages	Active PMN-elastase released from azurophil granula of neutrophil granulocytes
Role in inflammatory response	Primary factor in inflammatory response	Unknown	Contributes to processes of gut inflammation	Released from cells during cell activation	Infiltrates the mucosa during intestinal inflammation
Stability in feces	Up to 5 days at room temperature	For 2 days at room temperature	Up to 7 days at room temperature	Up to 7 days at room temperature	Up to 3 days at 2–8°C
Diseases in which increased levels of markers have been reported	IBD	Cancers (including colorectal, pancreatic, gastric, renal, lung)	Inflammatory disorders (rheumatoid arthritis and cystic fibrosis)	IBD	Inflammatory reactions involving neutrophils e.g., chronic joint inflammation, bacterial infection
Role as a marker for predicting relapses	Promising[22]	Unknown	Unknown	Potential to predict relapse in IBD	Potential tool for the differential diagnosis of IBD[27]

Abbreviations: PMN, polymorphonuclear neutrophils. Information derived from: Judd *et al.* Journal of gastroenterology and hepatology 2011; 26(10):1493–1499 and Schröder *et al.* Alimentary Pharmacology & Therapeutics 2007;26:1035–1042

Table 2. Proposed interpretation of results of a rapid point-of-care test

Fecal calprotectin cut-off levels in semi-quantitative test				
100 μg/g	250 μg/g	400 μg/g	1000 μg/g	Interpretation
Patients in primary care with suspected IBS				
NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	Result confirms IBS*
POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	Result suggests IBS*, repeat in 6 weeks to confirm initial result
POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	Result suggests possible IBD, repeat in 4 weeks
POSITIVE	POSITIVE	POSITIVE	NEGATIVE	Result consistent with IBD, refer for specialist opinion
POSITIVE	POSITIVE	POSITIVE	POSITIVE	Result indicates IBD
Patients in primary care with established UC				
POSITIVE	POSITIVE	POSITIVE	POSITIVE	Result confirms active disease, consider changing/escalating treatment, consider admitting patient to hospital
POSITIVE	POSITIVE	POSITIVE	NEGATIVE	Result suggests flare of IBD, consider changing/escalating treatment
POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	Result suggests IBD in remission
POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	Result suggests IBD in remission
NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	Result confirms IBD in remission

IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; UC, ulcerative colitis

*In conjunction with Rome III criteria for IBS being met

Statement of Interests

All authors of this paper have served as an advisory board member for Tillotts Pharma

- Gerhard Rogler has served as a consultant for Abbott Switzerland and Abbott International, Tillotts Pharma, to FALK Germany, to Essex/MSD Switzerland, Novartis, Roche, and Vifor Switzerland. He has received speaker honoraria from Abbott, FALK, MSD, Tillotts Pharma, UCB, and Vifor. He has received educational grants and research grants from Abbott, Ardeypharm, Essex/MSD, FALK, Flamentera, Novartis, Tillotts Pharma, UCB and Zeller
- Alain Schoepfer has served as a speaker or a consultant for Tillotts Pharma and has received research funding from Tillotts Pharma, Abbott Switzerland, MSD Switzerland and UCB Switzerland. He has received educational grants and research grants from Abbott, MSD, UCB, Falk and AstraZeneca
- Ulrich Mittmann has served as a speaker and as consultant for Tillotts Pharma AG
- Morten H. Vatn has served as a speaker or consultant for Tillotts Pharma, Falk Foundation, Abbott, UCB and Schering Plough and has received research funding from Centocor, IPSEN, Ferring, Tillotts, Novartis and MEDA
- Anders Lasson has served as a speaker or consultant for Tillotts Pharma, Abbott Scandinavia AB, AstraZeneca, Ferring Pharmaceuticals and MSD
- Taina Sipponen has served as a speaker for Tillotts Pharma, Abbott, MSD and Roche

This study was supported by Tillotts Pharma.

Editorial support was provided by Jayne Blanshard of PHOCUS Services Ltd, a member of the Fishawack Group of Companies, and supported by Tillotts Pharma.